

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
5 February 2004 (05.02.2004)

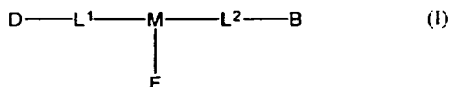
PCT

(10) International Publication Number
WO 2004/011556 A1

- (51) International Patent Classification⁷: **C09B 23/08**, G01N 33/58
- (21) International Application Number: **PCT/GB2003/003196**
- (22) International Filing Date: **28 July 2003 (28.07.2003)**
- (25) Filing Language: **English**
- (26) Publication Language: **English**
- (30) Priority Data:
0217556.0 30 July 2002 (30.07.2002) GB
10/241,333 11 September 2002 (11.09.2002) US
- (71) Applicant (for all designated States except US): **AMERSHAM BIOSCIENCES UK LIMITED [GB/GB]**; Amersham Place, Little Chalfont, Buckinghamshire HP7 9NA (GB).
- (72) Inventor; and
- (75) Inventor/Applicant (for US only): **COTTON, Graham [GB/GB]**; Albachem Limited, Elvington Science Centre, By Gladsmuir, Lothian EH13 1EH (GB).
- (74) Agents: **HAMMER, Catriona, MacLeod et al.**; Amersham plc, Amersham Place, Little Chalfont, Buckinghamshire HP7 9NA (GB).
- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:
— with international search report
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2004/011556 A1

(54) Title: **SITE-SPECIFIC LABELLING OF PROTEINS USING CYANINE DYE REPORTERS**



(57) Abstract: Disclosed are compounds of formula (I): in which D is a dye selected from a cyanine dye or a derivative thereof; B is an affinity tag; F comprises a target bonding group selected from a carboxylic acid thioester group and a 1,2-aminothiol group; M is a group adapted for attaching to F; and L¹ and L² each independently comprise a group containing from 1 to 40 linked atoms selected from carbon atoms which may optionally include one or more groups selected from -NR¹-, -O-, -CH=CH-, -CO-NH- and phenylenyl groups, where R¹ is selected from hydrogen and C₁-C₄ alkyl. The invention also relates to methods that afford direct attachment of the cyanine dye reporter group to either the N-terminus or C-terminus of a synthetic or recombinant peptide or protein, and their derivatives, in a site-specific manner, coupled with purification of the resultant labelled molecule.